DETECTION OF MYCOBACTERIUM ON GROWTH MEDIA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional patent application Ser. No. 62/976,631, filed 14 Feb. 2020, the contents of which are herein incorporated by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to methods and compositions used in detecting bacteria.

BACKGROUND OF THE INVENTION

[0003] The Problem of *Mycobacterium*

[0004] *Mycobacterium* is a genus of acid-fast bacteria that encompasses approximately 200 species. This genus includes serious human pathogens such as the causative agents of tuberculosis and leprosy. While *Mycobacterium tuberculosis* and *Mycobacterium leprae* are more globally known and studied, another category of *Mycobacterium,* called non-tuberculous *Mycobacterium* (NTM), is emerging as a significant threat to public health. In some places, NTM infections cause a greater disease burden than tuberculosis.

[0005] NTM exist ubiquitously in most environments and have recently gained interest as a frequent cause of infection. NTM infections most commonly lead to pulmonary disease; other possible infections include lymphadenitis, skin infections, and disseminated disease. Importantly, immunocompromised individuals are far more susceptible to NTM than most individuals. Treatment for mycobacterial infections are often lengthy, expensive, and extremely harsh on the patient, therefore early detection and prevention are imperative as control measures.

[0006] NTM infections have not been shown to transmit person to person. Most infections thus far have been traced to the environment, predominantly from water through aerosol inhalation and aspiration. Alarmingly, many clinical cases have been traced to potable water systems, including municipal drinking water and hospital water.

[0007] Limitations of Growth Media and Current Diagnostics for *Mycobacterium*

[0008] Currently, the most commonly used media for the growth of *Mycobacterium* are Lowenstein-Jensen (LJ) media, R2A media, and Middlebrook media (including 7H9, 7H10, and 7H11). Culture plates remain the "gold standard" for identifying mycobacterial infections or contamination (ASTM, 2015). Since water and/or clinical samples from which *Mycobacterium* are isolated are frequently contaminated with other bacteria, and since *Mycobacterium* grow much slower than average bacteria, isolating them from samples can be difficult as current media offer little differentiation and selection for them (Radomski et al., 2010).

[0009] Diagnostics for *Mycobacterium,* especially NTM, are severely limited, expensive, and time-consuming. The standard *Mycobacterium* diagnostics include spread-plating a sample onto a limited nutrient agar plate, often with antibacterial ingredients to inhibit non-mycobacterial growth and allow for easier selection of the bacteria.

[0010] Current decontamination or pretreatment steps utilized to isolate *Mycobacterium* from overgrown samples involve harsh reagents and complicated procedures that can significantly inhibit the growth of multiple species of *Mycobacterium.* For example, Cetylpyridinium chloride (CPC) is widely used to decontaminate water samples to aid in the isolation of *Mycobacterium.* Recent studies show that sample pretreatment of CPC can significantly reduce the growth of clinically important *Mycobacterium* species such as *M. abscessus.* Another pretreatment used on samples is the reagent N-acetyl-1-cysteine-sodium hydroxide (NALC-NaOH). Pretreatment with this reagent requires a complex protocol (i.e. time-consuming incubations and centrifugations) that can take up to over an hour per sample.

[0011] What is required are improved techniques and products for detecting and differentiating *Mycobacterium.*

SUMMARY OF ONE EMBODIMENT OF THE INVENTION

Advantages of One or More Embodiments of the Present Invention

[0012] The various embodiments of the present invention may, but do not necessarily, achieve one or more of the following advantages:

[0013] the ability to differentiate *Mycobacterium* from other bacteria;

[0014] the ability to detect non tuberculous *Mycobacterium* (NTM);

[0015] provide a novel growth medium for *Mycobacterium;*

[0016] provide a pretreatment method for enhancing positive detection of *Mycobacterium;*

[0017] provide a more cost-effective method for detection of *Mycobacterium.*

[0018] These and other advantages may be realized by reference to the remaining portions of the specification, claims, and abstract.

Brief Description of One Embodiment of the Present Invention

[0019] In one aspect of the present invention, there is provided a method for determining the presence of *Mycobacterium* in a sample. The method may include obtaining a sample from the environment. A portion of the sample may be plated onto a growth medium and incubated for an incubation period. After the incubation period, an inspection of one or more bacterial growth colonies may determine the presence of *Mycobacterium* in the environment. The growth medium may comprise an agar based growth medium comprising agar, one or more amino acid and nitrogenous supplementation elements, one or more trace elements and vitamins, one or more carbon sources, one or more neutralizing agents, and crystal violet. The crystal violet may be provided in an amount in excess of 0.5 μg/ml.

[0020] In one embodiment, the crystal violet may be provided in an amount in excess of 1.0 μg/ml. In one embodiment, the crystal violet may be provided in an amount in excess of 1.5 μg/ml. In one embodiment, the crystal violet may be provided in an amount in excess of 2.0 μg/ml.

[0021] In one embodiment, the sample may be treated with sodium dodecyl sulfate containing glycine hydrochloride prior to plating.

[0022] **8** In one aspect, there is provided a method for determining the presence of *Mycobacterium* in a sample. The method may include obtaining a sample from the